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Effect of pentobarbital on pH and electrolyte levels after induced seizure in rats

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Summary We studied the effects of high doses of pentobarbital (PB) and carbamazepine (CBZ) on electrolyte levels and pH in an epileptic animal model. Pentobarbital decreased Ca^{2+} and Na^{+} levels without pentylenetetrazole (PTZ). After this, Ca^{2+} and Na^{+} levels continued to decrease except when CBZ was used, which preserved the Ca^{2+} levels PTZ may have opposed effects on PB. Our results suggest that PB causes changes in electrolyte levels and pH, but these changes are diminished by CBZ.
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Introduction

One of 11 people has epilepsy problems, experiencing at least one seizure at some points. The tendencies to have recurrent, unprovoked seizures occur with a prevalence of about 0.5%, and a cumulative lifetime prevalence of 3%. It cover different conditions with varying etiology.[1] Epilepsy is not a disease, but a syndrome of different cerebral disorders of the central nervous system, which is characterized by paroxysmal, excessive, and discharges of large numbers of neurons.[2]

The conditions grouped under the term epilepsy constitute an area of continuing medical need. It has been estimated that about 20% of the patients with epilepsy using the first generation of antiepileptic drugs (pentobarbital, phenytoin, carbamazepine, sodium valproate and diazepam).[1]

Carbamazepine (CBZ) is one of the most effective and regularly used antiepileptic drugs. This substance is effective in to manage various types of epilepsy, including partial and generalized tonic–clonic seizures.[1]

Pentobarbital is a major drug in the control for canine, feline, and human epilepsy and can significantly reduce the severity of seizures. PB raises the threshold for seizure discharge and inhibits the initiation, diffusion, and spread of discharge from

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the neural focus. Blood-level PB is clinically effective 12—24 h after oral administration; it is metabolized by the liver and extracted by the kidney. The common side effects of PB are ataxia, sedation, polyuria, polydipsia, polyphagia, and it may depress both respiratory drive and mechanisms responsible for the rhythmic character of respiration.[2]

Pentobarbital is a known inducer of microsomal enzymes (cytochrome P-450 (CYP), NADPH-cytochrome P-450 reductase, NADPH oxidase, glutathione-*S*-tranferase), which are responsible for the metabolic breakdown of a large number of endogenous and exogenous chemical compounds.[2]

Studies in the last few years suggest that body electrolytes may be involved in some types of epilepsy and may increase the recurrence of seizures. Reports point to the vital role of body electrolytes sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) for seizure conditions to develop. No abnormality was noticed in serum Na^+ in epileptic patients during and after a seizure. With unaltered Na^+ level and hypokalemia patients, increase in recurrence of seizures was noted. That changes in Ca^{2+} levels are responsible for beginning convulsions is well documented.[3]

Epilepsy is characterized by recurrent seizures occurring overlong periods of time, kindled seizures are achieved by electrical or chemical stimulation. Examples of chemicals produce kindling in rats include pentylenetetrazole (PTZ), FG-7142, and picrotoxin.[4]

Pentylenetetrazole induces prototypical systemic seizure in epileptic rodents. The different susceptibility of the cortical and subcortical structures to PTZ-induced drastic effects may be related to their gamma aminobutyric acid type A ($GABA_A$) receptor density.[5,6] Cognitive disorders were shown to change with neuronal loss in the hippocampus. The animal models offer an opportunity to examine changes associated with epilepsy.[7,8]

PTZ-induced convulsions are linked with abnormal electrical recordings of brain activity. The beginning of seizures by PTZ is attributed to repress $GABA_A$ receptor Cl-channel, which thus attenuates GABA-dependent inhibition[9] and/or activation or *N*-metyl-D-aspartate (NMDA) receptors.[10—12]

GABA may mediate its synaptic events through two types of receptors—ionotropic and metabotropic. Among ionotropic receptors associated with a chloride channel so called $GABA_A$ and $GABA_C$ receptors are distinguished—metabotropic ones linked to the cascade of second intraneuronal messengers are $GABA_B$ receptors. $GABA_A$ receptor complex consists of a number of binding sites for GABA itself, benzodiazepines, barbiturates, ethanol and picrotoxin which is a chloride channel blocker. When GABA binds to its recognition site on the $GABA_A$ receptor complex, an opening of the chloride channel occurs with the subsequent influx of chloride anions into a neuron, resulting in its hyperpolarization.[3]

Therefore, in this research, we directly proposed to evaluate the effect on the levels of some electrolytes (Ca^{2+}, Na^+ and K^+), and pH, and HCO_3 levels after seizures are induced in animal subjects. Two different anesthetics, PB and ketamine and xylazine (KX) mixture and CBZ, were employed to compare the impacts of PB in an attempt to establish an adequate epileptic animal model and a good clinical method to study and monitor animals in laboratory experiments.

Methods

Animals

Twenty adult male Wistar rats (mean body weight, 448.8 g) were used as subjects in this study. Rats were housed five in a cage under a 12-h light/12-h dark cycle; food and water were available and administered ad libitum. Animals were treated according to international standards on animal handling. The procedure was approved by the Ethic and Investigation Committees of the National Rehabilitation Institute of Mexico City.

The rats were divided into three groups:

Group 1: Rats ($n = 5$) were anesthetized with PB 90 mg/kg i.p. They received a dose of 90 mg/kg body weight PTZ (Sigma, USA) dissolved in 0.9% saline solution i.p.
Group 2: Rats ($n = 5$) were anesthetized with PB 90 mg/kg i.p. They received a dose of 50 mg/kg body weight of CBZ i.p. and posteriori 30 min 90 mg/kg body weight PTZ i.p., dissolved in 0.9% saline solution.
Group 3: Rats ($n = 5$) were anesthetized with a mixture of Ketamine 80 mg/kg and Xylazine 8 mg/kg IM. They received a dose of 90 mg/kg body weight PTZ i.p. dissolved in 0.9% saline solution.

Blood samples (400—500 μl) were taken from the heart by cardiac puncture under anesthesia at two different times: first, basal register previously at PTZ and CBZ administration, and the second an hour after PTZ injection; the number of serial bleeds did not exceed 1%[13] of the rat's body weight; syringes were heparinized with 1000 UI/mL of blood to get electrolytes and do arterial blood gas measurements.

pH, bicarbonate (HCO_{3a}), sodium (Na), potassium (K) and ionized calcium (ICa) were measured by a blood gas analyzer (GEM mod. premier 3000).

Data analysis

For the experiments using blood gas and electrolytes as variables, all values were expressed as mean $\pm$ standard error (S.E.). Results were analyzed by the *U*-Mann–Whitney test (SPSS for windows, release 10.0). In all cases, the probability of error less than 0.05 were selected as the criterion for statistical significance from control values.

Results

During the study, we observed a mortality of 25%; the final number of sample subjects was 15 rats ($n = 15$). To compare PB effects on electrolytes, we used another anesthetic in this study: the KX mixture. We took basal results with anesthetic mixture as normal values. Then, we analyzed the differences between Groups 1 and 2 with basal samples and noticed significant differences in pH, Na^+ and Ca^{2+} ($p \leq 0.047$, 0.035 and 0.009) (Table 1).

The next comparison was done with the treatment involving PTZ administration after 1 h among groups given PB and KX. There were significant differences in pH, Na^+ and Ca^{2+} ($p \leq 0.016$, 0.009 and 0.009), respectively (Table 1).

Then, we compared the basal response of animals in Group 3 with those exposed to the PTZ treatment. In this case, there were no significant differences seen, except when we compared Group 1 basal and after PTZ changes in Ca^{2+} (Table 1).

The rats in Groups 1 and 2 did not present apparent seizures, waking up 5 or 10 min after PTZ. Only those in Group 3 had seizures for 2 h about 2 min after PTZ administration.

Discussion

The main purpose of this experiment was to investigate pentobarbital release in the pH and electrolyte levels before and after induced seizure in rats. Second, we employed pentobarbital rather than ketamine and xylazine (KX) mixture to investigate if there are differences between the drugs effects because we certainly consider that, the pentobarbital is better anticonvulsant and anesthetic compared with the ketamine and xylazine mixture.

The PTZ model of epilepsy, not only produces epilepsy like seizure activity, but also mimics seizure-induced cognitive and emotional changes that are similar to what is observed in human epilepsy, particularly those with temporal lobe foci,[9,14] that's why this is a good model of epilepsy study.

The results of this study showed an increase of Ca^{2+} extracellular levels in Ketamine anesthetized animals, suggesting that it causes an inhibiting depolarizing response due to the Ca^{2+} in the NMDA receptors. For this argument, we took these issues as normal values to compare and assess PB effects on pH and electrolyte levels.

Ketamine is an anesthetic agent known to block NMDA receptors, blocked T-type Ca^{2+} current.[15]

The body electrolytes play an essential role for enabling seizure conditions to development; and routine laboratory estimation of serum Na^+, K^+, Mg^{2+}, and Ca^{2+} is essential for the rational understanding and management of epileptic patients.[16]

PB increased the pH values (significance of $p \leq 0.047$) and increased Na^+ ($p \leq 0.035$), opposite Ca^{2+} levels; this electrolyte decreased in Groups 1

Table 1 pH and electrolyte levels basal and after Pentylenetetrazol (PTZ) induced seizure; Pentobarbital (PB), Ketamine and Xilazine (KX), Carbamazepine (CBZ)

Variable	Group 1 PB + PTZ		Group 2 PB + CBZ + PTZ		Group 3 KX + PTZ	
	Basal	1 h	Basal	1 h	Basal	1 h
pH	7.42 ± 0.02 [a]	7.40 ± 0.02 [b]	7.36 ± 0.02	7.39 ± 0.01	7.34 ± 0.02	7.29 ± 0.01
Na	147.20 ± 3.14 [c]	154.2 ± 2.71 [d]	150 ± 2.97	151.8 ± 1.71	139.2 ± 1.39	135.8 ± 1.77
K	3.8 ± 0.31	3.46 ± 0.20	4.04 ± 0.60	3.28 ± 0.13	3.54 ± 0.15	3.98 ± 0.26
Ca	0.99 ± 0.12 [e]	0.64 ± 0.06 [f,g]	0.73 ± 0.14	0.79 ± 0.06	1.84 ± 0.19	1.68 ± 0.23
HCO_3	23.1 ± 1.67	19.94 ± 1.45	18.9 ± 2.63	20.76 ± 1.18	20.42 ± 1.03	17.64 ± 1.7

Data are expressed as mean $\pm$ S.E.M. Mann–Whitney Test.
[a] Significant compared to basal group 3, $p = 0.047$.
[b] Significant compared to 1 h group 3, $p = 0.016$.
[c] Significant compared to basal group 3, $p = 0.035$.
[d] Significant compared to 1 h group 3, $p = 0.009$.
[e] Significant compared to basal group 3, $p = 0.009$.
[f] Significant compared to basal group 1, $p = 0.047$.
[g] Significant compared to 1 h group 3, $p = 0.009$.

and 2. This effect is important because PB facilitates the inhibition of synapses GABAergics, inhibiting Na^+ and Cl-channels. Investigators in 2003, indicate the immobilizing action and part the hypnotic action of pentobarbital is mediating by B3-containing $GABA_A$ receptors.[17]

In contrast with others barbiturates, which exert most if not all of their clinically relevant actions via B2- and B3-containining $GABA_A$ receptors, the PB has a wider range to targets, modulating the activity not only $GABA_A$ receptors,[18] but also of nicotinic acetylcholine receptors, AMPA receptors, kainate receptors and glycine receptors.[19]

Slobodan and Christoper[20] postulated importance of at least some T-Ca^{2+} currents in the action of some anticonvulsant drugs. All barbiturates and anaesthetics could produce complete blockade of T-Ca^{2+} current in rat.

Many documents show that a low-level of Ca^{2+} is responsible for the initiation of convulsions[9,21]; in this job, PB declined Ca^{2+} levels, probably because it is a good anticonvulsive drug. With the KX mixture, Ca^{2+} levels were higher than those with PB.

This response with KX explains why rats have seizures with this anesthetic when we put PTZ. However, with PB, rats did not have the seizures and woke up after being given PTZ.

Studies have shown that severe epileptic seizures lead to acute cerebral metabolic and circulatory changes. Besides systemic changes such as acute hypertension results from epileptic seizures, glucose, having a fundamental importance in brain oxidative metabolism, is increasingly carried and consumed in the brain during epileptic seizures, as a result of seizure activity.[12,22]

But, in this situation, we had changes in electrolytes and pH, only with PB. When we used KX, changes in these parameters were not observed.

By exchanging intracellular H^+ for extracellular Na^+, PB performs a fundamental role in various cell roles, including pH control, volume homeostasis, and cell growth,[23] and can significantly lessen seizure severity rate. PB raises the threshold for seizure discharge and inhibits the beginning, diffusion, and spread of discharge from the neural focus.[24]

The apparent contrasts in pH, Na^+ and Ca^{2+}, found an hour after PTZ, are likely to be a direct consequence of PB use. That are why it is considered the middle life of this medicine.

In this course, PTZ could lead to epileptic seizures without interfering greatly with metabolism.[25] In this particular situation, except in a chronic model, these results could change.

Group 2 presents values similar to those shown by Group 1, except that Ca^{2+} levels did not change in Group 1 due to CBZ effects. CBZ works to block changes in Ca^{2+}, inhibited Ca^{2+} and Na^+ channels.

CBZ blocks sustained repetitive firing in individual neurons: this drastic effect is caused by the blockade of voltage-dependent Na^+ or Ca^{2+} channels.[16] In this study, CBZ preserved Ca^{2+} levels in rats after PB and PTZ. It is important to know what happened with the neurotransmitter in these cases because, in rats with PB and CBZ, when we put the PTZ i.p., the rats woke up and did not have seizures. Probably PTZ is opposing to PB.

These results indicate that drugs such as PB creates changes in systemic electrolytes and pH but these changes could be stopped with other drugs like CBZ. This work is important because it gives an adequate animal model for the study of epilepsy, reveals the position of anesthetics in this category of work and gives alternating perceptions on how to use electrolyte parameters to know the effect of these drugs.

The results reflect the PB is a good anticonvulsive drug for this decreasing in Ca^{2+} levels, to prevent the induced seizures by PTZ in rats.

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